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Study on the Susceptibility of Rats to Various Strains of Mycobacteria

Report II. Histological Examination of Rats Infected with Various Strains of Mycobacteria

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INTRODUCTION

A previous paper reported the results obtained by a quantitative cultivation technique regarding the growth of mycobacteria in the organs of rats, and gross organ changes induced by infections with these bacilli. In infections with virulent tubercle bacilli (H37Rv strain and bovine RM strain), the multiplication of bacilli in organs was marked after a while. But the bacilli ceased to multiply after two to four weeks, and then decreased in number. Considerable tubercle formation, which later decreased in size and number, was noted only in the lungs of rats. In other organs almost no gross changes were seen.

In infection with more attenuated strains (BCG, H37Ra and a variant of H37Rv resistant to isoniazed) slight multiplication of bacilli was observed in the lung, but this was very transitory and soon decreased. Almost no gross changes other than the formation of a few nodules were noted.

From these results it is concluded that the albino rat has much more native resistance to tuberculosis than the rabbit and the guinea pig, and is also more resistant than the hamster.

In this paper histological changes found in the organs of rats infected with these mycobacteria will be reported, and the difference in the histological responses to mycobacteria between rats and other more susceptible animals will be discussed.

MATERIALS AND METHODS

Tissue materials were obtained from animals used in the previous experiments. Five types of tubercle bacilli were used : the human type H37Rv strain, bovine RM strain, BCG strain, H37Ra strain and a variant of H37Rv resistant to 50γ isoniazid (Catalase negative). These have already been described. The dosages and intervals of time when the infected rats were killed have also

been given in the previous paper. For histological examination sections were stained with haematoxylin and eosin, by the Ziel-Heidenhaim method, and with rodamin-auramin for fluoromicroscopic examination of tubercle bacilli.

EXPERIMENTAL OBSERVATION

1) Changes after infection with H37Rv strain. (Table 1)

Table 1. Histological Changes after Infection with H37Rv Strain.

Organ	Lung					Spleen		Kidney		Liver	
Lesion	Tissue Reaction				Bacilli	Tissue Reaction	Bacilli	Tissue Reaction	Bacilli	Tissue Reaction Fatty Degeneration	Bacilli
Time after Infection	Tubercle	Large Exudate Cells	Giant Cells	Alveolitis							
4 W	++	++	+++	+++	++++	++	−	−	−	+++	−
	++	+++	++	++	+++	++	−	−	−	+++	+
6 W				++	−	−	−	−	−		
	++	++	+++	+++	+++	+	−	−	−	+++	+
8 W	+	++	−	+++	++++			−	−	+++	−
	+	++	+	++	++++			−	−		−
10 W		+++	+++	+++	++++	+++	−	−	−	+	+
						++	−	−	−		
16 W		+	+	++	−	−	−	−	−	−	+
	++	+++		+++	++	−	−	−	−	−	−
6 M		+		+	−			−	−	−	−
10 M				+	−						

Lung : Although changes were more marked than in other organs of rats, they were definitely slighter than those observed in the rabbit or guinea pig. Four weeks after infection, epithelioid cell tubercles were seen in considerable number (Fig. 1), but they were conspicuously less in number and size than tubercles found in rabbits' or guinea pigs' lungs infected with the same bacilli. The tubercles were composed of epithelioid cells rich in nuclear chromatin, lymphocytes and in the center of the tubercles were scattered a small number of neutrophiles or their nuclear debris. Giant cells containing relatively small numbers of nuclei rich in chromatin were seen (Fig. 2), but no necrosis and caseation was noted anywhere. Alveolar walls were thickened with cell infiltration. In the alveolus there was slight infiltration by large exudative cells. At the sites of tubercles and cell infiltrations a very large number of acid fast bacilli were seen. No change was noted in the bronchi or in the blood vessels.

These changes indicated in all rather a type of foreign body inflammation.

Almost the same histological changes in the lung as just described continued until the end of 16 weeks, except for some modifications according to the individual differences of the rats used.

After six months, inflammatory tissue changes were scarcely seen and there was only proliferation of connective tissue. This may suggest that at the sites of previous lesions scar formation has progressed.

In all periods of observation (the latest was at 10 months) no caseation of tissue was noted at all.

Bacillary findings in tissues almost paralleled the tissue damage and the largest number of acid fast bacilli were seen in the 8th to 10th week. After the 10th week, however, bacilli began to decrease in number. After six months no bacilli were detected by staining. These tubercle bacilli were seen phagocytized in either the large exudate cells, the epithelioid cells or the giant cells, especially in the last (Fig. 3).

Spleen : The spleen showed histological changes next marked to those in the lung. From the fourth to the tenth week small epithelioid cell tubercles and giant cells having a type of foreign body giant cell were seen in the spleen (Fig. 4), and no necrosis and caseation was noted. In the 16th week no histological change was found in this organ. Bacillary stainings were missed by technical

Table 2. Histological Changes after Infection with Bovine RM Strain.

Organ	Lung				
Lesion	Tissue Reaction				Bacilli
Time after Infection	Tubercle	Large Exudate Cells	Giant Cells	Alveolitis	
3 W	+	+		+	+
4 W				+	+
	+			+	+
6 W				++	+
				++	−
8 W	+	++		++	−
				+	−
10 W				++	−
	+	+++		+	−
12 W	+	+++		+++	−
	+	++		+++	+
15 W				++	−
				+++	−

failure, in all instances.

Kidney : No visible change appeared throughout the observation period, and no bacilli were detected.

Liver : From the fourth to the eighth week peripheral fatty degeneration was observed in the liver (Fig. 5), but this change decreased after that and at 16 weeks no pathological findings were noted. Bacilli in the liver were apparently less in number than those in the lung.

2) Changes after infection with bovine RM strain. (Table 2)

Histological changes were examined up to the 15th week after infection.

Lung : In general tissue changes, including the formation of tubercles, exudation of large exudate cells to alveolus and cell infiltration in the alveolar septi etc. (Fig. 6), were slighter than those described in the above section. Almost all of these tissue changes disappeared in the 15th week, and very slight alveolitis and slight lymphocytic infiltration around the bronchi were seen. Bacilli stainable in the tissue were very small in number and inconstant. After the 8th week no bacilli were detected.

Almost no histological changes was seen in the other organs throughout the observation period.

3) Changes after infection with either H37Ra or resistant variant of the H37Rv strain. (Table 3, 4)

Table 3. Histological Changes after Infection with H37Ra Strain.

Organ	Lung					Spleen		Kidney		Liver	
Lesion	Tissue Reaciton				Ba-cilli	Tissue Reac-tion	Ba-cilli	Tissue Reac-tion	Ba-cilli	Tissue Reac-tion / Fatty Degen-eration	Ba-cilli
Time after Infection	Tu-bercle	Large Exu-date Cells	Giant Cells	Alve-olitis							
1 W				++	−	−	−	−	−	+++	−
2 W				+	−	−	+	−	−	+++	−
4 W								Hyper-amia	−	++	−
		+		++	−	−	−	Hyder-amia	−	+++	−
8 W	+		+	+	−	Small Necro-sis of White Pulp	++			+	+
				++	−			−	−	++	−
10 W		+		++	+		−		−	+++	−
	+		++	++	−		−		−	−	−
4 M				+	−		−		−	−	−
				++	−		−		−	+	−

Table 4. Histological Changes after Infection with Resistant Variant of H37Rv Strain.

Organ	Lung					Spleen		Kidney		Liver	
Lesion	Tissue Reaction				Bacilli	Tissue Reaction	Bacilli	Tissue Reaction	Bacilli	Tissue Reaction	Bacilli
Time after Infection	Tubercle	Large Exudate Cells	Giant Cells	Alveolitis						Fatty Degeneration	
4 W	−			+	+	−	−	−	−	##	−
					##	+	−	−	−	###	−
6 W		+		+	−		−		−	###	+
		+		+	−	−	−	−	−	###	−
8 W	+	+	+	##	##	Atrophy of White Pulp		−	−	##	−
				##	−		−	−	−	###	−
10 W								−	−		
						+	−	−	−		
16 W		+		##	##	−	−	−	−	−	+
	+	##		##	+	−	−	−	−	+	−
6 M	+	+	+	##	−					+	−

These two strains are in general thought to have the least virulences against susceptible laboratory animals, but in the rat histological changes induced by these bacilli were almost the same as those found in infection with bovine RM strain.

Lung : In the lung infected with H37Ra, small but well-defined tubercles were seen from the 8th to the 10th week. After the 8th week until the 6th month at the maximum there were small tubercles in the lung infected with a resistant variant of the H37Rv strain (Fig. 7). Bacilli in the lung infected with H37Ra, however, were positive only in one case after 10 weeks, and were completely negative in all other specimens. In infection with a resistant variant of H37Rv strain bacilli in the lung were positive from the 4th to the 16th week.

Spleen : In the spleen almost no change was noted, excepting that slight atrophy and small necrosis of the white pulp or catarrhal inflammation in the sinuses were seen in a few cases.

Kidney : There were no changes other than hyperemia in the medulla of kidneys infected with H37Ra at 4 weeks. No bacilli were detected.

Liver : In both groups in early stage, considerable peripheral fatty degeneration was noted, but there were no changes after 8 to 10 weeks. Bacilli were also negative in this organ.

4) Changes after infection with the BCG strain. (Table 5)

Table 5. Histological Changes after Infection with BCG Strain.

Organ	Lung					Spleen		Kidney		Liver	
Lesion	Tissue Reaction				Bacilli	Tissue Reaction	Bacilli	Tissue Reaction	Bacilli	Tissue Reaction Fatty Degeneration	Bacilli
Time after Infection	Tubercle	Large Exudate Cells	Giant Cells	Alveolitis							
1 W		+			−			−	−	++	−
2 W				+	+			−	−	+++	−
				+	−			−	−	+++	−
4 W				+	−			−	−	++	−
		++	++	+	−			Hyperamia	−	+++	+
6 W				+	−			Hyperamia	−	±	−
				+	−			Hyperamia	−	+	−
8 W				+	−			−		++	−
				++				−		+++	−
10 W				++	−			−	−	+	−
				+	−			−	−	++	−
4 M				+	−	−	−		−	+	−
				±	−			−	−	++	−

Changes induced by infection with the BCG strain were the least in the present experiments.

Lung : In only one case exudation of large exudate cells and foreign body giant cells were seen in the 8th week, and very slight alveolitis was noted in other cases (Fig. 8). There were only two specimens of the second week in which tubercle bacilli were detected. All other specimens were negative.

Kidney : There were no changes except slight hyperemia at 4 to 6 weeks.

Liver : Slight fatty degeneration was seen until the 4th month.

In summary, the greatest changes were found in the lung, and next the spleen. There were almost no tuberculous changes in the liver and kidney. In the liver apparent fatty degeneration induced by the toxic effect of inoculated bacilli was seen. The H37Rv strain was the most virulent of the strains used. Bovine RM was next most virulent, a variant of H37Rv was the third, H37Ra the fourth, and BCG strain the least.

DISCUSSION

Histological examination shows that tissue changes induced by infection with various strains of tubercle bacilli in rat's lungs are similar to those found in

other susceptible animals, accompanying the formation of epithelioid tubercles, infiltration of large exudate cells and the formation of giant cells. Tubercle bacilli are found numerously in these cells, especially in the giant cells. Large exudate cells in this report corresponds to the foamy cells, defined by either Wessels[1], Yamazaki[2], Wada[3], Kusamitsu[4] or Sabin[5] in their reports. Wessels pointed out that these cells were surely observed in the lung, but not in the other organs. Epithelioid cells observed in the rat are also very similar to those found in other suscepible animals, but chromatin is richer in the rat than in the others. Giant cells found in the rat's organs are relatively small, having less than ten nuclei. These cells are not of the Langhans's type but rather a type of foreign body giant cells, as has also been pointed out by Wessels. These tuberculous changes were not so apparent in the other organs of rats.

Caseation is one of the outstanding phenomena of tuberculosis in the susceptible animal. In the rat, however, although in general the cellular response of the host to the invading parasite is much the same, at no time is there necrosis of cells. This particular character of rat's tissue, lack of caseation, may by very significant in the high native resistance of this animal to tuberculosis. It seems that this characteristic may have an intimate relationship with the lack of response to tuberculin allergy.

The BCG strain has a somewhat curious behavior in the rat. Although this strain is in general thought to have stronger virulence than the H37Ra strain, multiplication of bacilli and tissue changes in the organs were apparently less in the rat infected with the BCG strain. Furthermore, as shown in a subsequent report, the BCG strain is capable of growth in the body fluid of rats, when it is cultivated *in vivo* by the chamber method, notwithstanding the impossibility of the other virulent strains to grow. A similar phenomenon was observed in the hamster by Tao[6]. It may be difficult to explain clearly this discrepancy in multiplication of the BCG strain in whole tissue and in body fluid. However, it may suggest that the behavior of multiplication of BCG in the cells and in the body fluid is not uniform with the behavior of other virulent strains.

SUMMARY

Histological examination of the organs of rats infected with various mycobacteria (H37Rv, bovine RM, BCG, H37Ra and variant of H37Rv resistant to isoniazid) were performed at various intervals until after six months. Among organs of rats the lung is most susceptible to all strains used, and the spleen is next most. The liver and kedney are almost nonsusceptible. Among the strains used the H37Rv has the strongest virulence, and the bovine RM the next strongest. The BCG strain was the weakest. This order was apparently shown

in the histological examinations.

Histological changes induced by infection are similar to tuberculous changes found in other susceptible animals, ...epithelioid tubercles, infiltration of large exudate cells, and formation of giant cells. A unique and very important difference is the lack of necrosis or caseation in the rat.

These tissue changes developed for a while, and then ceased to develop, and at last, several months after the infection, disappeared completely or left a small amount of scar tissue.

The author wishes to express her sincere thanks to Dr. Y. Kobara for the assistance in carrying out this work.

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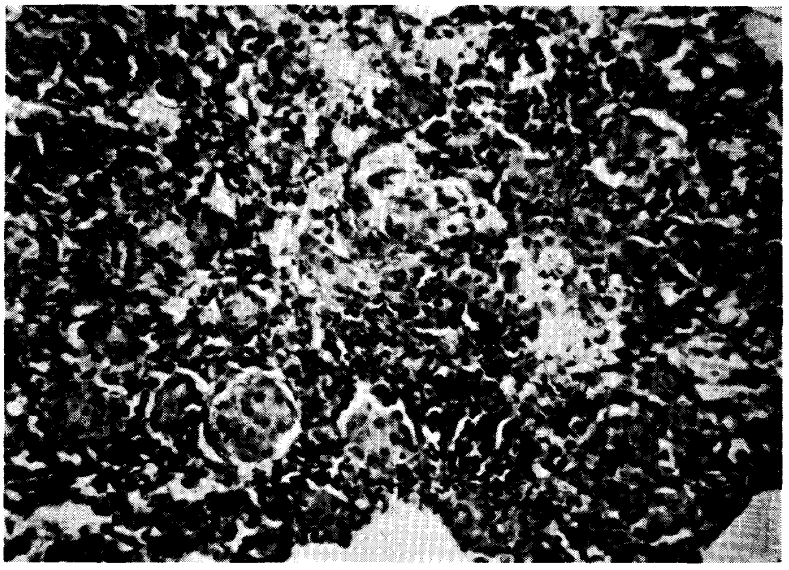


Fig. 1. Small tubercles chiefly composed of giant cells and epithelioid cells. The lung of an albino rat sacrificed four weeks after intravenous injection of H37Rv strain. (100×)

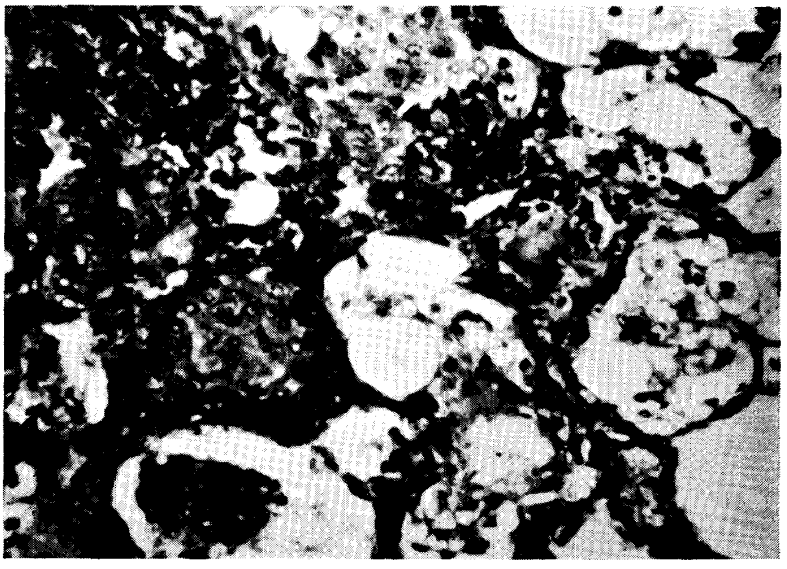


Fig. 2. Giant cells and degenerative large exudative cells in the peripheral layer of the tubercles. The lung of an albino rat sacrificed four weeks after injection of H37Rv strain. (200×)

Fig. 3. Acid-fast bacilli (which were found) in the giant cells. (Hematoxylin and eosin staining). The lung of an albino rat sacrificed 46 weeks after injection of H37Rv strain. (900×)

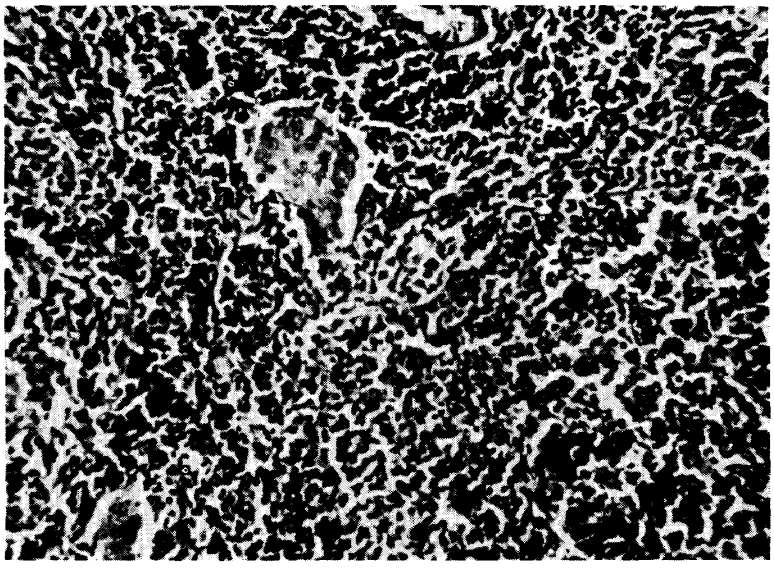


Fig. 4. Obvious sinus catarrh and giant cells. The spleen of an alblno rat sacrificed four weeks after injection of H37Rv strain. (100×)

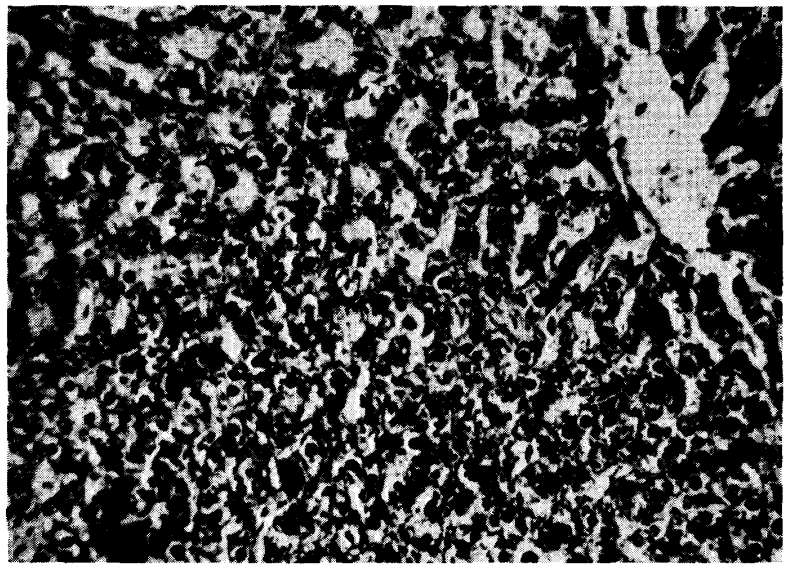


Fig. 5. Peripheral fatty degeneration in the liver of an albino rat sacrificed four weeks after injection of H37Rv strain. (100×)

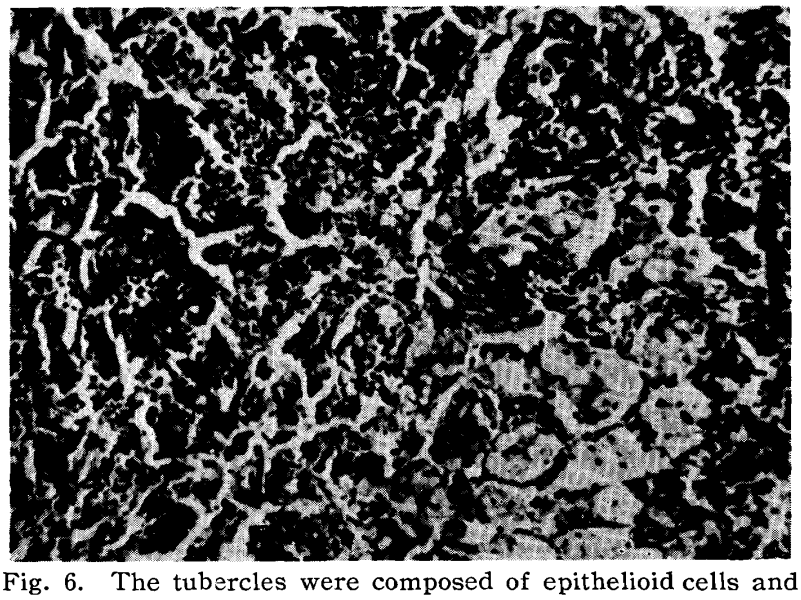


Fig. 6. The tubercles were composed of epithelioid cells and large exudative cells. The lung of an anlbino rat sacrificed five weeks after injection of bovine RM strain. (100×)

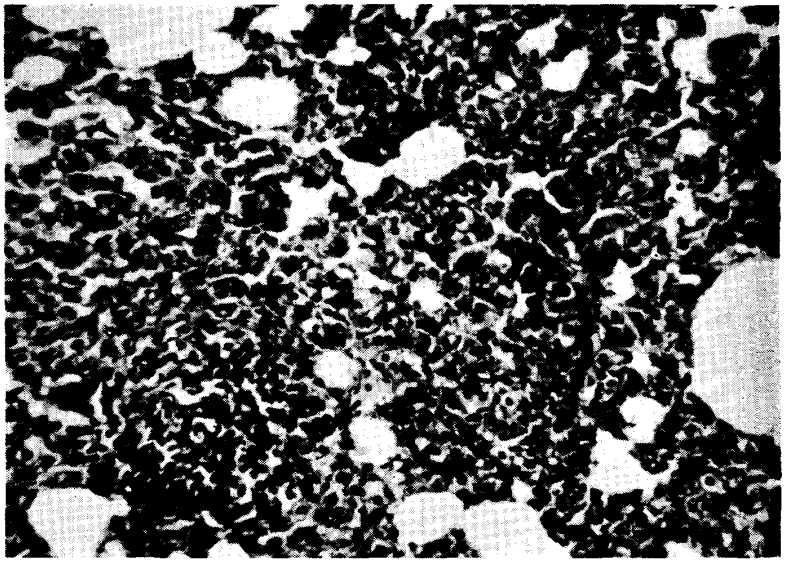
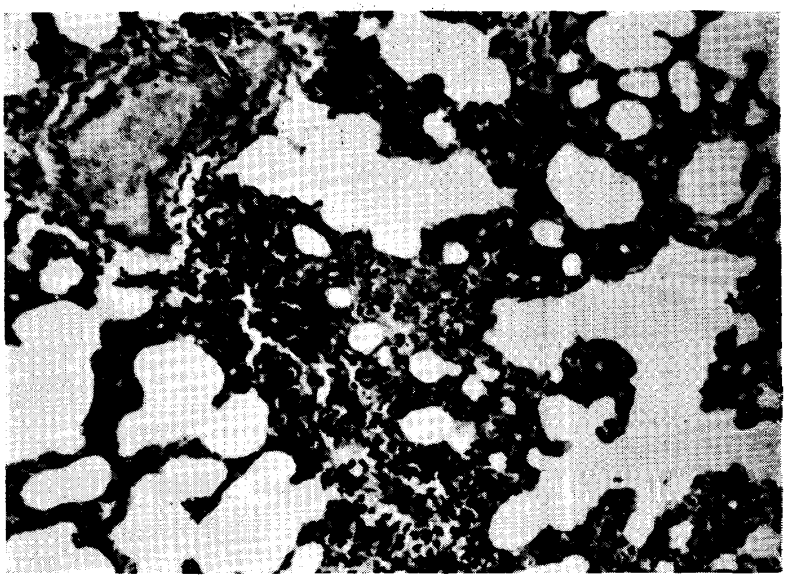


Fig. 7. Small tubercles and cell infiltration and hypertrophy of alveolar walls. The lung of an albino rat sacrificed four weeks after injection of variant of H37Rv resistant to isoniazid. (100×)



Eig. 8. Cell infiltration and hypertrophy of alveolar walls. The lung of an albino rat sacrificed four weeks after injection of a BCG strain. (100×)